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Relationship of Vitamin D Binding Protein Polymorphisms and Lung Function in Korean Chronic Obstructive Pulmonary Disease

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Purpose: Multiple genetic factors are associated with chronic obstructive pulmonary disease (COPD). The association of gene encoding vitamin D binding protein (VDBP, *GC*) with COPD has been controversial. We sought to investigate the types of *GC* variants in the Korean population and determine the association of *GC* variants with COPD and lung function in the Korean population. **Materials and Methods:** The study cohort consisted of 203 COPD patients and 157 control subjects. *GC* variants were genotyped by the restriction fragment-length polymorphism method. Repeated measures of lung function data were analyzed using a linear mixed model including sex, age, height, and pack-years of smoking to investigate the association of *GC* genetic factors and lung function. **Results:** *GC1F* variant was most frequently observed in COPD (46.1%) and controls (42.0%). *GC1S* variant (29.0% vs. 21.4%; p=0.020) and genotype 1S-1S (8.3% vs. 3.4%; p=0.047) were more commonly detected in control than COPD. According to linear mixed model analysis including controls and COPD, subjects with genotype 1S-1S had 0.427 L higher forced expiratory volume in 1 second (FEV_1) than those with other genotypes (p=0.029). However, interaction between the genotype and smoking pack-year was found to be particularly significant among subjects with genotype 1S-1S; FEV_1 decreased by 0.014 L per smoking pack-year (p=0.001). **Conclusion:** This study suggested that *GC* polymorphism might be associated with lung function and risk of COPD in Korean population. *GC1S* variant and genotype 1S-1S were more frequently observed in control than in COPD. Moreover, *GC1S* variant was more common in non-decliners than in rapid decliners among COPD.

Key Words: Vitamin D binding protein, polymorphism, lung function, chronic obstructive pulmonary disease

INTRODUCTION

The most critical risk factor for the development of chronic obstructive pulmonary disease (COPD) is cigarette smoking.[1] However, only 10–20% of heavy smokers develop airway obstruction and –20–30% of COPD patients never smoke.[2-6] Moreover, previous reports of familial aggregation of COPD suggest that genetic components are likely associated with susceptibility to the development of COPD.[7,8]

Numerous candidate genes have been reported to be associated with COPD. These genes are generally related to protease-antiprotease interaction, antioxidant effects, xenobiotic metabolism, and inflammation and immune response pathways.[9] One of the candidates involved in inflammation and immune reaction is the gene encoding vitamin D binding protein (VDBP, *GC*).

VDBP was first described by Hirschfeld in 1959 as a marker in gamma-globulin of human serum and characterized as a group-specific component.[10] In 1975, it was identified as the plasma protein that binds vitamin D.[11] The major role of VDBP is transporting 25-hydroxyvitamin D, the major circulating form of vitamin D, and 1,25-dihydroxyvitamin D, the most active vitamin D metabolite. However, when expressed by neutrophils, VDBP activates macrophages and augments monocyte and neutrophil chemotaxis.[12-15] These roles of VDBP may contribute to the chronic inflammatory response in COPD.

GC, located on chromosome 4, is approximately 42 kb in size and has more than 120 reported variants.[16] Most of the variants are rare except three commonly recognized variants according to single nucleotide polymorphisms at rs4588 and rs7041 in exon 11: *GC1F* (A/G), *GC1S* (A/T), and *GC2* (C/G).[17] The association of these VDBP polymorphisms and the risk for COPD has been debated in the literature for some time.[9,18-26] *GC2* tended to be protective variants for COPD in Caucasian populations and *GC1F* tended to be risk variants for COPD in Asian populations.[27] However, Asian studies were conducted in the limited ethnicities, and *GC* variants were not evaluated in South Korea. Moreover, the studies were conducted in a small number of patients and the relationship with lung function decline was rarely investigated.[20]

The purpose of this study was to evaluate the frequencies of *GC* variants in Koreans with COPD as well as healthy subjects. We determined that certain *GC* variants are associated with a genetic susceptibility to COPD in Koreans and examined the correlation between *GC* variants and lung function in COPD patients.

MATERIALS AND METHODS

Subjects

The COPD group consisting of 203 patients who were taken from the Korean Obstructive Lung Disease (KOLD) Cohort, in which patients with COPD or asthma have been enrolled from pulmonary clinics in 17 hospitals in South Korea from June 2005 to 2011, were selected for this study. The subjects were culled from a KOLD Cohort that fulfilled the following criteria: post-bronchodilator ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FEV_1/FVC) <0.7, over 40 years of age, smoking history of 10 or more pack-years, and no or minimal abnormality on chest radiography. The control group was selected from a community-based prospective cohort, South Korea. Among 2534 people recruited in 2006, 157 participants had a normal pulmonary function test, 10 or more pack-years of smoking history, and were over the age of 40. The study protocol was approved by the Institutional Review Boards of the 17 hospitals included in the KOLD Cohort and the community-based prospective cohort for control group, and informed written consent was obtained from all patients.

Pulmonary function testing

Spirometry was performed according to American Thoracic Society/European Respiratory Society guidelines. Airway obstruction is defined by Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria as FEV_1/FVC ratio of <0.7. The severity of the disease was based on the percent predicted FEV_1 in accordance with GOLD criteria.[28] For COPD patients, pulmonary function tests (PFTs) were performed at follow-up visits annually and PFT was performed at least two times in 178 (87.6%) with an average of 3.7 times during the study.

Genotyping

For genotyping *GC*, DNA was extracted from blood and polymerase chain reaction (PCR) was performed followed by restriction fragment-length polymorphism analysis. The region that includes the two point mutation sites in exon 11 (Glu/Asp 416 and Thr/Lys 420) was amplified.[27] We used upstream primer of 5'TAATGAGCAAATGAAAGAAG3' and downstream primer of 5'TGAGTAGATTGGAG-

TGCATAC3'.[20,25] The final PCR product was a fragment of 462 base pairs (bp).

PCR was performed in a thermal cycler (DNA Thermal Cycler; Perkin Elmer Cetus; Norwalk, CT, USA). PCR reactions were carried out in a volume of 40 μL containing 100 ng DNA, 1.5 mM $MgCl_2$, 10 mM Tris Cl (pH 8.3), 40 mM KCl, 4% dimethyl sulphoxide, 0.2 mL of each dNTP (Amersham Biosciences KK; Tokyo, Japan), 0.5 μmol/L of each primers, and 3.75 units of Taq DNA polymerase (Bioneer, Daejeon, Korea). After amplification, PCR products were digested with Hae III (Toyobo; Osaka, Japan), or Eco T14 I (Takara Bio; Otsu, Japan) at 37°C overnight. Hae III cuts the *GC1S* product into two bands of 295 bp and 167 bp, whereas Eco T14 I cuts the *GC2* product into two bands of 302 bp and 156 bp. *GC1F* PCR product remains uncut by either enzyme (Fig. 1).

Definitions

Rapid decliners were defined as those with a decrease in FEV_1 ≥3.0% predicted/yr and non-decliners as those with an increase in FEV_1 ≥0% predicted/yr.[24]

Data analysis

Differences in distribution and frequency of variants and genotypes among the groups were examined by chi-squared test. Normally distributed variables are presented as means ±standard deviations, and non-normally distributed variables are presented as medians (interquartile range). Genotype frequencies and Hardy-Weinberg equilibrium (HWE) for the *GC* between COPD and control groups were determined by chi-squared test. Statistical inference of the genotype effect on FEV_1 was conducted with the linear mixed model to consider correlation between annually measured FEV_1. Statistical inference of the genotype effect on FEV_1 at the 0.05 significance level was conducted with the linear mixed model to consider correlation between repeated measures. COPD patients and control subjects were included for linear mixed model and COPD indicator was included as dummy variable to explain their mean differences. We also found the heteroscedasticity between COPD patients and control subjects, and a different variance-covariance matrix was applied. The optimal correlation structure between repeated observations was selected with the likelihood ratio test based on the restricted maximum likelihood method and the unstructured correlation format was chosen.[29,30] Age, sex, height, pack-year, and their interactions were included as covariates and the significant interactions were selected with likelihood ratio test based on the maximum likelihood method. Marginal effects are all included in our final model. In all analyses, a p-value <0.05 was deemed to be statistically significant.

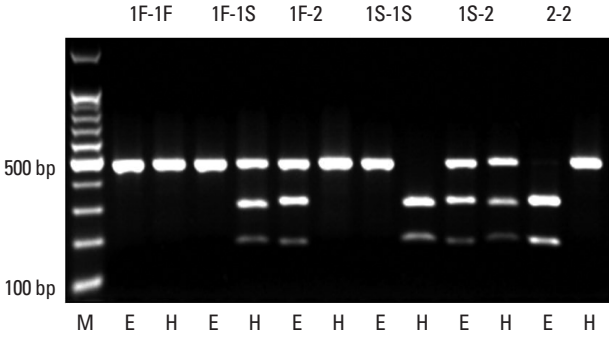

Fig. 1. Restriction fragment-length polymorphism analysis of *GC*. Eco T14 I cuts *GC2* into two bands of 302 base pairs (bp) and 156 bp, whereas Hae III cuts *GC1S* into two bands of 295 bp and 167 bp. *GC1F* remains uncut by either of the enzymes (E: digested by Eco T14 I, H: digested by Hae III).

RESULTS

Population characteristics

All baseline characteristics of the study population are summarized in Table 1. Both groups exhibited a similar gender distribution of significant male dominance. COPD patients were also of more advanced age than the control population. The number of current smokers was higher in controls compared to COPD group, whereas former smokers were more prevalent in the COPD group than controls. Among COPD patients, 17 (8.4%), 101 (49.8%), 73 (36.0%), and 12 (5.9%) patients were classified as GOLD class I, II, III and IV.

Genotyping

The frequency of each variant and genotype in COPD and control groups are compared in Table 2. Each of the investigated single nucleotide polymorphisms (rs4588 and rs7041) was in HWE in the controls (all p>0.05). Frequencies of *GC1F*, *GC1S*, and *GC2* variants in COPD patients were 46.1%, 21.4%, and 32.5%, respectively, and 42.0%, 29.0%, and 29.0%, in controls, respectively. *GC1S* variant was more frequent in controls than COPD patients (29.0% vs. 21.4%; p=0.020). There were no significant differences in sex ratio, age, and smoking history between the different types of genotypes. Genotype 1F-2 was most common in both groups and genotype 1S-1S was significantly more frequent in control than COPD (8.3% vs. 3.4%; p=0.047).

Table 1. Baseline Characteristics of the Study Population*

Characteristics	COPD (n=203)	Control (n=157)	*p* value
Sex, male	199 (98.0)	148 (94.3)	0.058
Age, yr	67 (62–71)	53 (47–59)	<0.001
Height, cm	165 (162–170)	167 (162–170)	0.571
Smoking status			
Current	55 (27.2)	75 (47.8)	<0.001
Former	147 (72.8)	82 (52.2)	
Pack-yrs	46.0±23.4	30.7±17.4	<0.001
FEV_1, L	1.42±0.49	3.17±0.59	<0.001
FEV_1, % predicted	53.1±16.9	93.9±12.8	<0.001
FVC, L	2.99±0.81	4.05±0.70	<0.001
FVC, % predicted	79.6±19.3	93.3±11.9	<0.001
FEV_1/FVC, %	47.5±10.9	78.3±5.1	<0.001

COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.
*Data are presented as numbers (percentages) for categorical variables. Continuous variables are presented as means±standard deviations or median (interquartile range).

Comparison between rapid decliners and non-decliners

Table 3 shows the comparison of characteristics and genotypes between 62 rapid decliners and 74 non-decliners. Baseline characteristics including sex ratio, age, and spirometry data were not different between the two groups. In comparing the variants and genotypes, *GC1S* variant was more frequently observed in non-decliners (26.4% vs. 16.1%, p=0.042) and genotype 1F-2 was more frequently observed in rapid decliners (37.1% vs. 21.6%, p=0.047).

Parameters associated with FEV_1 according to linear mixed model analysis

The difference in FEV_1 between different genotypes was investigated by linear mixed model analysis including sex, age, height, and pack-years of smoking as shown in Table 4. FEV_1 was 2.649 L lower in COPD compared to controls (p<0.001), 0.345 L lower in females than in males (p=0.014), and 0.033 L lower per year of age (p<0.001). Moreover, as stature increased by 1 cm, FEV_1 also increased by 0.027 L (p<0.001). Among the different genotypes, subjects with genotype 1S-1S had 0.427 L higher FEV_1 than those with other genotypes (p=0.029). The genotype/smoking pack-year interaction was found to be particularly significant among subjects with genotype 1S-1S; FEV_1 decreased by 0.014 L per smoking pack-year (p=0.001).

According to the linear mixed model analysis, the final equations were obtained as follows: FEV_1=0.488-0.033×age (year)+0.027×height (cm)-0.012×smoking pack-years +0.427 (for control group with genotype 1S-1S); FEV_1= 0.488-0.033×age (year)+0.027×height (cm)+0.002×smoking pack-years (for control group without genotype 1S-1S); FEV_1=0.488-0.013×age (year)+0.027×height (cm)-0.012×smoking pack-years-1.711 (for COPD group with genotype 1S-1S); FEV_1=0.488-0.013×age (year)+0.027×height (cm) +0.002×smoking pack-years-2.649 (for COPD group without genotype 1S-1S).

Table 2. Variant and Genotype Frequency of *GC* in COPD and Control Groups*

Variables	COPD (n=203)	Control (n=157)	*p* value
Variant			
GC1F	187 (46.1)	132 (42.0)	0.281
GC1S	87 (21.4)	91 (29.0)	0.020
GC2	132 (32.5)	91 (29.0)	0.309
Genotype			
1F-1F	45 (22.2)	34 (21.7)	0.907
1F-1S	41 (20.2)	31 (19.7)	0.915
1F-2	56 (27.6)	33 (21.0)	0.198
1S-1S	7 (3.4)	13 (8.3)	0.047
1S-2	32 (15.8)	34 (21.7)	0.199
2-2	22 (10.8)	12 (7.6)	0.304

COPD, chronic obstructive pulmonary disease.
*Data are presented as numbers (percentages).

DISCUSSION

The potential association of polymorphisms in *GC* with the risk of COPD and lung function was investigated for the first time in a Korean population. Subjects with *GC1S* variant and genotype 1S-1S were more commonly observed in control than in COPD. Moreover, *GC1S* variant was more frequent in non-decliners than in rapid decliners among COPD. According to the linear mixed model analysis, gen-

Table 3. Characteristics of Subjects with COPD Classified by Annual Rates of Decline in FEV_1*

Characteristics	Rapid decliners (n=62)	Non-decliners (n=74)	*p* value
Sex, male	61 (98.4)	73 (98.6)	-
Age, yr	65.5 (61–70)	67 (61–70)	0.896
Smoking status			
Current	13 (21.3)	26 (35.1)	0.078
Former	48 (78.7)	48 (64.9)	
Pack-yrs	44.7±18.1	49.9±28.5	0.197
FEV_1, L	1.58±0.49	1.52±0.54	0.482
FEV_1, % predicted	61.4±18.7	55.4±17.8	0.058
FVC, L	3.30±0.90	3.08±0.78	0.135
FVC, % predicted	88.2±23.3	80.4±19.3	0.036
FEV_1/FVC, %	48.5±10.3	48.9±11.1	0.798
Variant			
GC1F	57 (46.3)	67 (45.3)	0.860
GC1S	20 (16.1)	39 (26.4)	0.042
GC2	47 (37.9)	42 (28.4)	0.095
Genotype			
1F-1F	12 (19.4)	16 (21.6)	0.745
1F-1S	10 (16.1)	19 (25.7)	0.176
1F-2	23 (37.1)	16 (21.6)	0.047
1S-1S	2 (3.2)	3 (4.1)	-
1S-2	6 (9.7)	14 (18.9)	0.130
2-2	9 (14.5)	6 (8.1)	0.235

COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.
*Data are presented as numbers (percentages) for categorical variables. Continuous variables are presented as means±standard deviations or median (interquartile range).

otype 1S-1S showed higher initial FEV_1 than other genotypes. However, FEV_1 decreased more in 1S-1S patients than in other genotypes as smoking pack-years increased.

Frequencies of the different genotypes previously discussed vary based on ethnicity (Table 4). In the Japanese population, *GC1F* variant is most commonly observed, comprising approximately half of the population (0.58–0.61 in COPD and 0.48–0.49 in control). In Caucasians, *GC1S* variant is most common with a frequency of 0.53–0.61 in COPD and 0.58 in controls.[19,20] The Korean population in this study showed a similar distribution to the Japanese population.[18,25] *GC1F* variant was most frequent in COPD (0.46) and control group (0.42), although its frequency was smaller than that in a Japanese population.

Prior investigations have demonstrated different results regarding the association between polymorphisms in *GC* and risk of COPD; however, studies focusing on Asian populations have only been in Chinese and Japanese cohorts. Ishii, et al.[19] reported *GC1F* variant and genotype 1F-1F were significantly higher in COPD than in control (85.7% vs. 75.6%, p=0.036; 36.5% vs. 20.7%, p=0.035) in a Japanese population. In another Japanese study, Ito, et al.[20] reported a higher frequency of genotype 1F-1F in COPD patients than in controls (32% vs. 17%, p=0.014). In a Chinese population, Lu, et al.[23] suggested that *GC1F* variant and genotype 1F-1F were risk factors for COPD (55.8% vs. 40.4%, p=0.018; 33.3% vs. 11.5%, p=0.005). In a Canadian Caucasian population, Horne, et al.[18] reported *GC2* variant and genotype 2-2 were less frequently observed in a COPD group. Relative risks of genotype 2-2, 2-1S, 2-1F for COPD were 0.8, 0.7, and 0.5, respectively. Moreover, they stated that *GC1F* variant was a significant risk factor for COPD (relative risk=4.8). Schellenberg, et al.[25] were able to confirm that genotype 2-2 was protective against COPD (odds ratio=0.17, 95% CI 0.03–0.83). In an American population, Kueppers, et al.[31] also showed genotype 2-2 as a protective factor for COPD (relative risk=0.2). Despite all these reports, several studies have failed to show association between *GC* polymorphisms and COPD.[24,32]

Most of these studies were conducted with a cross-sectional design, such that only the frequencies of certain *GC* polymorphisms were compared between COPD and con-

Table 4. Frequency of *GC* Polymorphism and the Risk of Chronic Obstructive Pulmonary Disease in Other Countries

Country	Phenotype	No. of cases/ controls	Variant		COPD			Control		
			Risk	Protective	*GC1F*	*GC1S*	*GC2*	*GC1F*	*GC1S*	*GC2*
Caucasian										
Canada[25]	COPD	75/64	-	*GC2*	0.19	0.53	0.28	-	-	-
Canada[18]	COPD	104/413	-	*GC2*	0.19	0.61	0.20	0.12	0.58	0.3
USA[9]	COPD	127 families and 304/441	-	-	-	0.43	0.29	-	0.42	0.27
USA & Canada[24]	Rapid decline of FEV_1	279/305	-	-	0.14	0.55	0.31	0.14	0.57	0.29
USA & Canada[36]	High FEV_1 vs. Low FEV_1 among COPD	537/533	-	-	0.16	0.54	0.30	0.15	0.55	0.30
France[32]	Heavy smoker with high FEV_1 vs. never smoker with low FEV_1	45/43	-	-	0.17	0.60	0.23	0.21	0.46	0.33
Iceland[22]	COPD	112/183	-	-	0.09	0.61	0.30	0.10	0.62	0.28
	Chronic bronchitis	48/183	*GC1F*	*GC2*	0.22	0.60	0.18	0.10	0.62	0.28
Russia[21]	COPD	298/237	-	-	0.49	0.24	0.27	0.48	0.26	0.26
Asian										
Japan[20]	COPD	103/88	*GC1F*	-	0.58	0.22	0.20	0.49	0.27	0.24
	Rapid decline of FEV_1	86/21	*GC1F*	-	-	-	-	-	-	-
	Emphysema	85 COPD	*GC1F*	-	-	-	-	-	-	-
Japan[19]	COPD	63/82	*GC1F*	-	0.61	0.18	0.21	0.48	0.27	0.24
	DPB	82/82	-	-	0.43	0.29	0.28	-	-	-
China[23]	COPD	69/52	*GC1F*	-	0.56	0.24	0.20	0.40	0.29	0.31
Tartar[21]	COPD	298/237	*GC1F*	*GC2*	0.44	0.26	0.30	0.43	0.34	0.23
Korea (current)	COPD	203/157	-	*GC1S*	0.46	0.21	0.33	0.42	0.29	0.29
	Rapid decline of FEV_1	62/74	-	*GC1S*	0.46	0.16	0.38	0.45	0.26	0.29

COPD, chronic obstructive pulmonary disease; USA, United States of America; FEV_1, forced expiratory volume in 1 s; DPB, diffuse panbronchiolitis.

trol groups. A few studies demonstrated the relationship of *GC* polymorphisms with lung function decline through longitudinal analysis.[20,24] Although Sandford, et al.[24] reported no difference between fast decliners and non-decliners, Ito, et al.[20] showed that *GC1F* variant still is a risk factor for rapid lung function decline even in a longitudinal analysis. However, in the latter, the number of subjects with available follow-up lung function data was small and the follow-up duration of 1 year may not be long enough to accurately analyze lung function decline. The size of the subjects in our study is too small to demonstrate the effect of *GC* polymorphisms on COPD and lung function. However, we have analyzed all the follow-up lung function data of subjects with longer duration of follow-up using a linear mixed model.

There are several possible explanations for the association between *GC* polymorphisms and lung function decline. The major role of VDBP is to bind and transport vitamin D. Vitamin D is essential for maintaining normal bone growth and calcium homeostasis, but it also contributes to immune modulation. Epidemiologic research has demonstrated vitamin D deficiency is associated with many types of chronic disease and these chronic diseases are all comorbidities of COPD. Moreover, vitamin D deficiency is a risk factor of lung function decline in COPD through dysregulation of adaptive and innate immunity. Janssens, et al.[33] reported that vitamin D deficiency correlates with the severity of COPD and certain types of *GC* polymorphisms are associated with reduced vitamin D levels.[34] Moreover, VDBP enhances the chemotactic activity of C5-derived peptides on human neutrophils and monocytes. Enzymatic processing of the carbohydrate side-chain of VDBP transforms the molecule into a potent macrophage-activating factor (MAF). In turn, VDBP-MAF stimulates macrophage activity. Recently, Wood, et al.[35] reported that the *GC2* genotype is less efficient at converting VDBP to VDBP-MAF.

However, there are limitations to the present study. First, the sex and age between the two groups were not exactly matched. The COPD group was comprised of slightly more males and older subjects compared to the control group. Though the effect of age was adjusted for by statistical

methods, some of the younger control subjects may yet develop COPD in the future. Moreover, cautious interpretation of the results is needed because of the relatively small sample size, especially *GC1S* variant and genotype 1S-1S. Analysis of lung function in control subjects was limited because follow-up lung function data were only available in five subjects of control group. Finally, vitamin D status is also an important factor in investigating the association of *GC* genotypes and lung function, but it was not included in this study.

In conclusion, this study suggested that *GC* polymorphisms might be associated with COPD and lung function in Korean population. Subjects with *GC1S* variant and genotype 1S-1S were more common in control than in COPD. *GC1S* variant was more frequent in non-decliners than in rapid decliners among COPD. The subjects with genotype 1S-1S showed higher initial FEV_1 than other genotypes. However, genotype 1S-1S was associated with lower lung function in the context of cigarette smoking. As the amount of smoking in subjects with genotype 1S-1S increases, decrease in lung function became more pronounced. To elucidate the relationship of *GC* polymorphisms and lung function in COPD, additional studies with larger number of subjects are required.

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